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INVESTIGATION OF PHOTOCHEMILUMINESCENCE OF HUMAN BLOOD PLASMA

[Following is the translation of an article by Ye. G. Dontsova, S. A. Predtechenskiy, and I. I. Sapezhinskiy, Institute of Chemical Physics, USSR Academy of Sciences, Moscow, published in the Russian-language periodical Biofizika (Biophysics) Vol XIII, No 6, 1968, pages 1048-1053. It was submitted on 4 Aug 1967.]

The photochemiluminescence of solutions of human blood plasma is conditioned mainly by proteins; the greatest contribution in luminescence is made by serum albumin. In the investigated samples of blood plasma the activators and extinguishers of chemiluminescence are absent. In plasma there are no perceptible quantities of acceptors of protein free radicals. Data were obtained on the regularities of free radical processes which take place in UV-irradiated plasma.

A study of the chemiluminescence of solutions of biopolymers which have been induced by various types of radiations makes it possible in a number of cases to obtain valuable information concerning free radical reactions which lead to the destruction of macromolecules. On the basis of investigations conducted [1-5] it is possible to make up a concept concerning the processes which lead to the appearance of prolonged afterglow in solutions of proteins. Under the influence of UV-light peroxide free radicals of proteins are formed. The important role of tryptophan in the appearance of radicals has been established [2]. Free radicals of protein enter into numerous reactions [4], one of which (disproportionation of radicals) is accompanied by the emergence of products in an electron-excited state. On the basis of the mechanism which was established in work [1] it is possible to obtain a formula connecting the basic parameters of afterglow

$$I = \eta \cdot \varphi \cdot I_0(1-10^{-D})(1-e^{-k_1\tau})\cdot e^{-k_0 t}, \qquad (1)$$

where I - intensity of afterglow at moment of time t; η - yield of chemiluminescence; φ - effectiveness of formation of free radicals; I_0 - intensity of irradiating light; D - optical density of irradiated solution, proportional to the concentration of protein in the solution; k_1 - constant of rate of growth of luminescence; τ - time of irradiation; k_0 - constant of rate of afterglow; t - time after termination of irradiation.

In the event of the presence in the system of substances of activators or extinguishers of luminescence there is a change in the yield of luminescence η without a change of remaining parameters [6]. With an increase in the effectiveness of formation of radicals (for example, due to the presence of sensitizers) there is an increase of φ. Following the introduction of acceptors of free radicals (or inhibitors of free radical processes) there is an increase in the constant for rate of decrease of luminescence [4].

However, up until now the studies have been made of photo-roentgeno-chemiluminescence of solutions of individual substances, therefore the investigation of photochemiluminescence of blood plasma, a complex system consisting of many dozens of components, is of great interest. On the one hand this may produce information about destructive processes taking place under actual biological conditions; on the other, such a system yields to modeling.

We attempted to answer the following questions: 1) What components of plasma are responsible for photochemiluminescence; what is the contribution of individual fractions in luminescence? 2) Does blood plasma contain substances which influence the yield of luminescense (activators, extinguishers)? 3) Does the composition of blood plasma contain acceptors of radicals or sensitizers? 4) What are the regularities of free radical processes which are taking place in blood plasma during irradiation?

The experiments were set up on a flow-type unit for the recording of weak chemiluminescence. The solutions (5 or 6 ml) under study were irradiated in a thermostatic vessel ($t = 25^{\circ}$) and then transfered to a measuring cuvette which was located close to the photocathode of an FEU-36 photomultiplier. The photocurrent was amplified and on an EPP-09 potentiometer recordings were made of the kinetic curves of afterglow. Irradiation of the solutions was done with a PRK-4 mercury lamp (in the majority of cases for 10 s). In the work we used dry human blood plasma and its component protein fractions; the solutions were prepared on a 0.1 M phosphate buffer, pH 7.0. On the basis of data obtained by the method of paper electrophoresis, and a determination of the percent content of protein (biuret method) the protein content of one of the samples of dry plasma was determined: albumin - 31.3%; α-globulin - 2.9%; β-globulin - 10.9%; γ-globulin - 12.4%. In preliminary tests a recording was made of the afterglow of native blood plasma of mice and solutions of various samples of dry human blood plasma.

Figure 1 shows the kinetic curve of photochemiluminescence of a solution of human blood plasma. As is apparent from the drawing, under the influence of UV-light a chemiluminescence develops which lasts for 15-20 minutes after irradiation. Kinetic curves of afterglow are characterized by the following values: 1) by the value of the constant for the rate of the first order of drop in luminescence

(in Fig. 1 the semilogarithmic anamorphosis of the curve is given (for this curve the magnitude of the constant is equal to $1.2 \cdot 10^{-3}$ s^{-1})); 2) by the magnitude of the light total, determining the overall number of acts of recombination leading to afterglow (determined by suspension of a paper figure, limited by the axis of abscissas and kinetic curve); 3) by the relative intensity 300 s after irradiation (in comparison with the internal standard, the "standard signal").

Each experimental point represents the result of 4-6 tests.

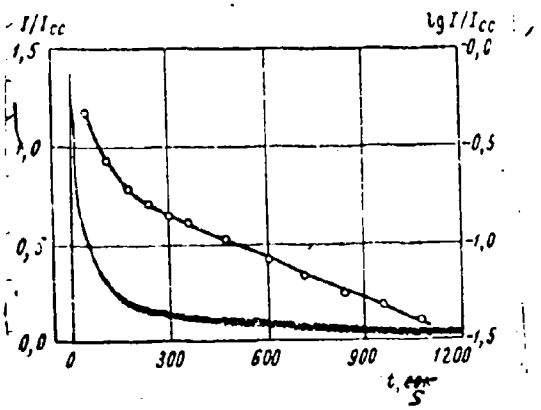

Fig. 1. Kinetic curve of photochemiluminescence of a 0.3% solution of human blood plasma and the semilogarithmic anamorphosis of this curve.

Blood plasma includes both high- and low-molecular compounds. The high-molecular compounds include mainly proteins (the content of nucleic acids and polysaccharides is insignificant). It can be assumed that luminescence is caused by the protein components of plasma. For clarification of this experiments were made on the separation of blood plasma by means of dialysis (10 ml of 7.5% blood plasma was dialyzed against 40 ml of phosphate buffer, pH 7, for 24 hours). As a result two solutions were obtained, in one of which there were proteins in a quantity equivalent to 1.5% plasma, and in the other - low-molecular substances, also equivalent in concentration to 1.5% blood plasma. Figure 2 shows the kinetic curves of photochemiluminescence of a 1.5% solution of whole plasma (1), and of high-molecular (2) and low-molecular (3) fractions. It is apparent from the drawing that luminescence of plasma is conditioned mainly by the protein fraction. If one were to accept the value of light total of whole plasma as 100%, then the share of the protein fraction comprises 79.1%, and low-molecular - 2.5%. The total of values is around 82%. The small non-coincidence (18%) of values for light total of plasma and components may be explained both by methodical causes (binding of a certain amount of protein by membrane) and by the presence in the low-molecular fraction of a small amount of sensitized substances.

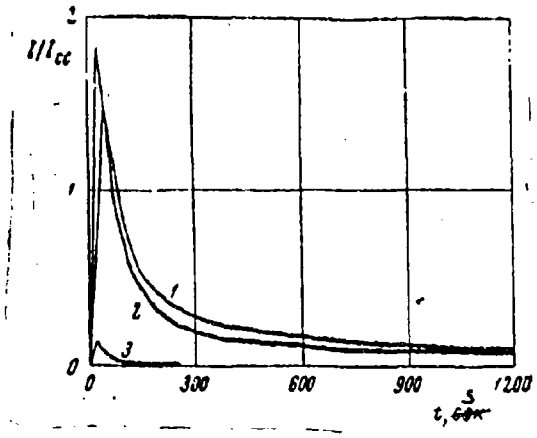

Fig. 2. Afterglow of a 1.5% solution of blood plasma (1), its high-molecular (2) and low-molecular (3) fractions.

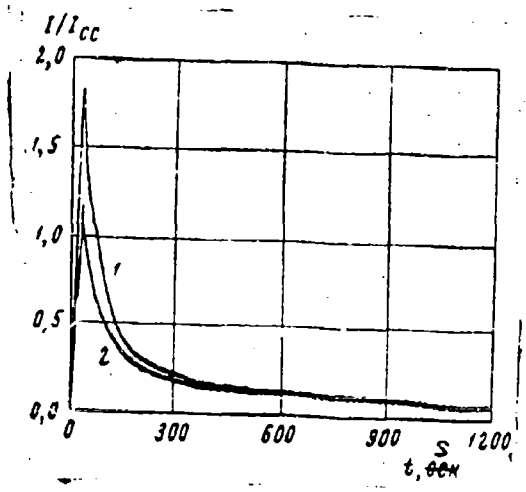

Fig. 3. Photochemiluminescence of 1.5% plasma (1) and a model solution of proteins equivalent in composition to plasma (2).

If the afterglow of plasma is caused by proteins, then it can be assumed that the luminescence of a model solution of proteins, equivalent in composition to plasma, will not differ strongly from the chemiluminescence of whole plasma. A model solution was prepared which had in its composition serum albumin, α-, β-, and γ-globulins in concentrations equivalent to 1.5% blood plasma. Figure 3 shows the kinetic curves of afterglow of a model solution (2) in a comparison

with a solution of 1.5% plasma, (1). It is clear from the drawing that the intensity of luminescence of blood plasma and model solution is sufficiently close. If one were to accept the value of light total for plasma as 100%, then in the model solution this value equals 81.1%. The difference is apparently caused either by an inaccuracy of electrophoretic analysis, or by the minor influence of low-molecular components, or by the fact that the model solution did not contain all the proteins of plasma.

For clearing up the contribution of separate proteins in photochemiluminescence of blood plasma, kinetic curves of afterglow were obtained for five proteins in concentrations corresponding to their content in 1.5% blood plasma. If one were to disregard the mutual screening of various proteins during absorption of UV-light, then on the basis of values of light totals it is possible to estimate the contribution of each protein in luminescence, taking the total of all light totals as 100%. The contribution of serum albumin comprises 77%, α-globulin 7.9%, β-globulin 8.4%, γ-globulin 5%, and fibrinogen 1.5%.

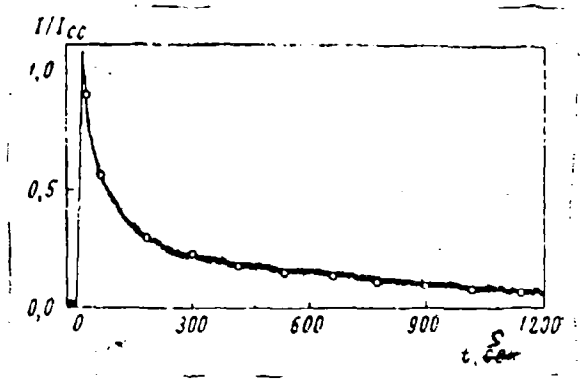

Figure 4. Influence of the addition of plasma on the photochemiluminescence of a solution of serum albumin.
Afterglow of 0.25% protein - solid curve; circles - afterglow of protein in the presence of 1.5% plasma, added after irradiation.

In order to clear up if plasma contains any substances which influence the yield of luminescence (extinguishers or activators) the following experiments were set up: an 0.25% solution of serum albumin was irradiated for 10 s. Immediately after irradiation a solution of blood plasma was added to it. If the composition of plasma included activators of luminescence, then the intensity of luminescence of the solution of protein would increase, if it contained extinguishers then it would decrease. Figure 4 shows the curves of afterglow of a solution of serum albumin (solid curve) and the same solution, but with the addition of plasma so that its concentration in solution comprised 1.5%. It is clear from the drawing that these curves coincide, i.e.,

the plasma in no way influences the afterglow of serum albumin. On the basis of these tests it can be asserted that in the investigated samples of blood plasma activators and extinguishers are absent.

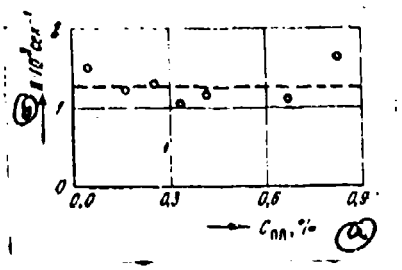

Figure 5. Dependence of the constant of rate of drop in luminescence of an 0.25% solution of serum albumin on the concentation of plasma added after irradiation.
Key: (a) C, plasma; (b) $K \cdot 10^3 s^{-1}$.

Preservation of the form of the kinetic curve indicates that in the composition of plasma there are no acceptors of protein free radicals. Experiments were made on the influence of additions of plasma of various concentrations on the constant of the rate of drop in the luminescence of serum albumin.

These results are presented in Fig. 5. It can be seen that within the limits of experimental scattering the magnitude of the rate constant does not change, which supports the assumption of the absence in plasma of perceptible amounts of acceptors of free radicals (inhibitors of free radical processes).

Thus, on the basis of the tests conducted it can be considered that the luminescence of plasma is caused by proteins, and there is an absence of influences (activation, extinguishing, reactions of acceptors) which complicate the picture of afterglow. Therefore it can be proposed that the data, characterizing the free radical processes in irradiated blood plasma, will be comparable with the data obtained earlier for individual proteins [1, 3, 5]. We studied the main kinetic characteristics of afterglow of blood plasma.

Figure 6, A shows the dependence of relative intensity of afterglow (300 s after irradiation) on the concentration of plasma. It is apparent that with an increase of concentration there is an increase of intensity, at first rapidly and then more slowly.

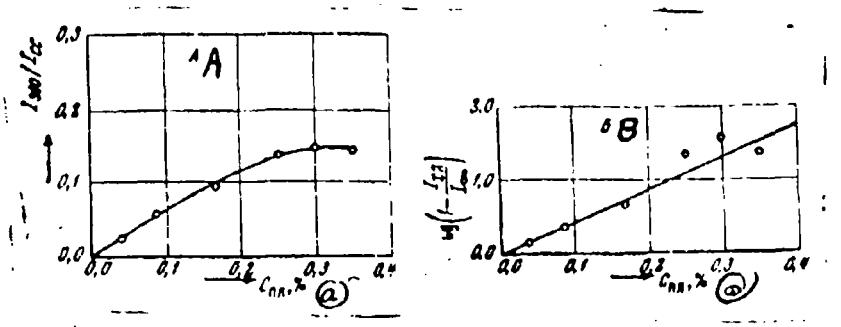

Fig. 6. Change in the magnitude of relative intensity of afterglow of a solution of blood plasma depending on concentration (A) and the anamorphosis of this curve (B).
Key: (a) C, plasma, %.

It can be proposed that the increase of luminescence is connected with an increase in the share of absorbed light. If this is so, then on the basis of formula (1) it is possible to obtain

$$I = I_\infty (1 - 10^{-D}), \qquad (2)$$

where I_∞ - intensity of luminescence with complete absorption of irradiated light. From here it follows that

$$\lg\left(1 - \frac{I}{I_\infty}\right) = -D.$$

Optical density D is directly proportional to concentration. Therefore if it is imagined that the data of Fig. 6, A are in coordinates "lg (1 - I/I∞) - concentration," then a linear dependence should be fulfilled. Such an anamorphosis is shown in Fig. 6, B. It is clear that the relationship (2) is fulfilled satisfactorily.

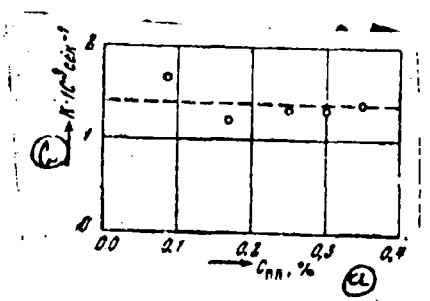

Figure 7. Dependence of the rate constant of photochemiluminescence of solutions of plasma on concentration.
Key: (a) C, plasma, %; (b) $K \cdot 10^3 s^{-1}$.

Figure 7 shows the dependence of the rate constant of drop in luminescence on the concentration of the plasma. From the figure it follows that the magnitude of the rate constant is not changed within the limits of experimental scattering. The average value of the rate constant comprises $1.4 \cdot 10^{-3}$ s^{-1}.

The dependence of the intensity of luminescence on the time of irradiation (the experiments were conducted with solutions of 0.5% plasma) represents a typical curve of increase of luminescence which is analogous to the curves obtained for serum albumin /1/. For a comparison studies were made of the dependences of the parameters of luminescence on concentration for one of the proteins of blood plasma - human serum albumin. It turned out that the intensity of luminescence increases with concentration of protein and the relationship (2) is satisfied.

Thus on the basis of kinetic experiments it follows that during the irradiation of blood plasma protein free radicals emerge. Satisfactory fulfillment of relationship (1) indicates that the limiting stage is a process of the first order of regrouping of free radicals /1/. Introduction of cysteine and propyl gallate into an irradiated solution of plasma leads to an increase in the constant of rate of drop of luminescence. Addition of eosine yellowish /6/ increases the intensity of luminescence by 10 times.

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